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(21) International Application Number: PCT/FI91/00240 (22) International Filing Date: 8 August 1991 (08.08.91) (30) Priority data: 905402 1 November 1990 (01.11.90) FI (71) Applicant (for all designated States except US): OY ALKO AB [FI/FI]; Salmisaarenranta 7 H, SF-00180 Helsinki (FI). (72) Inventors; and (75) Inventors/Applicants (for US only) : ROSSI, Marianne [FI/FI]; Pohjolaantie 16 B 2, SF-04230 Kerava (FI). LINKO, Yu-Yen [FI/FI]; LINKO, Pekka [FI/FI]; Otakallio 2 B 16, SF-02150 Espoo (FI). VAARA, Timo [FI/FI]; Itäinen Puistotie 16 C 14, SF-00140 Helsinki (FI). TURUNEN, Marja [FI/FI]; Raisiontie 7 A 3, SF-00280 Helsinki (FI).		(74) Agent: PAPULA REIN LAHTELA OY; Box 981, SF-00101 Helsinki (FI). (81) Designated States: AT, AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH, CH (European patent), CI (OAPI patent), CM (OAPI patent), CS, DE, DE (European patent), DK, DK (European patent), ES, ES (European patent), FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), GN (OAPI patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LK, LU, LU (European patent), MC, MG, ML (OAPI patent), MN, MR (OAPI patent), MW, NL, NL (European patent), NO, PL, RO, SD, SE, SE (European patent), SN (OAPI patent), SU ⁺ , TD (OAPI patent), TG (OAPI patent), US. Published <i>With international search report.</i>
(54) Title: OLIGOSACCHARIDE MIXTURE, AND PROCEDURE FOR ITS AFTER-TREATMENT (57) Abstract A procedure for after-treatment of an oligosaccharide mixture, wherein glucoamylase and/or yeast is made to act on an oligosaccharide mixture which has been obtained by making cyclomaltodextrin-gluconotransferase (CGTase, E.C.2.4.1.19) act on starch in the presence of acceptor sugar. By this procedure a novel oligosaccharide mixture lower in calories and less cariogenic than before is obtained.		

+ DESIGNATIONS OF "SU"

Any designation of "SU" has effect in the Russian Federation. It is not yet known whether any such designation has effect in other States of the former Soviet Union.

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OLIGOSACCHARIDE MIXTURE, AND PROCEDURE FOR ITS AFTER-TREATMENT

The present invention concerns a procedure for
5 after-treatment of an oligosaccharide mixture according
to the patent application FI 904124, and a mixture
obtained by means of said procedure.

It is known in the art to use α amylase in
order to liquefy starch-based, viscous mixtures and to
10 lower their viscosity. Hydrolysis of starch with
 α amylase gradually leads to production of maltose. In
the British Patent GB 2,019,406 commercial α and β amy-
lases are employed to lower the viscosity of 'coupling
sugar' syrups. In the Japanese Patent JP 76 73,140,
15 β amylase is used to decompose oligosaccharides pro-
duced from invert sugar or glucose isomerization mix-
tures, maltose being obtained as yield.

It is known in the art to use glucoamylase for
hydrolyzing starch, whereby starch breaks up to glu-
20 cose. Commercial glucoamylase preparations are mostly
composed of several different amylases (in: Starch,
Chemistry and Technology (1984), Eds. R.L. Whistler,
J.N. BeMiller and E.F. Paschall, Academic Press Inc.,
London, England). As disclosed in the British Patent
25 GB 3,660,236, glucoamylase is produced by moulds of
genus *Aspergillus*, such as *Aspergillus niger*, *Aspergil-
lus awamori*, and by some moulds of genus *Rhizopus* and
Endomyces. In the Japanese Patent JP 63 216,492, gluco-
amylase and/or commercial yeast has been used in con-
30 nection with producing neotrehalose and 2'- α -glucosyl-
maltose, for removal of the hydrolysis products formed
of starch, and free glucose.

In the procedure constituting the object of
the present invention, in the after-treatment is used,
35 differing from the procedures cited above, either solu-
ble or immobilized glucoamylase and/or yeast. Since,
moreover, the oligosaccharide mixture after-treatment

method of patent application FI 904124, with glucoamylase and/or yeast, has not been heretofore described, the procedure of the invention is a completely novel after-treatment method.

5 In the after-treatment procedure of the invention, glucoamylase is made to act on an oligosaccharide mixture as specified in patent application FI 904124, whereby the long-chain oligosaccharides are broken up to shorter units, and glucose is set free in the solution. The free glucose is eliminated with baking yeast from an oligosaccharide mixture according to patent application FI 904124 or from a glucoamylase-treated mixture. Proceeding in this manner, one obtains a novel oligosaccharide mixture which is lower in calories and less cariogenic. The characteristic features of the invention are presented in the claims and are hereby included in the disclosure section.

 The object of the invention is a procedure for after-treatment of an oligosaccharide mixture according to patent application FI 904124, and a mixture obtained by this procedure. In the after-treatment procedure of the invention, glucoamylase is made to act on an oligosaccharide mixture according to patent application FI 904124, whereby the long-chain oligosaccharides, particularly those with DP (glucose polymerizing grade) higher than 5, are broken down to shorter units and glucose is set free in the solution. The glucoamylase-treated oligosaccharide mixture contains in the first place oligosaccharides with DP between 3 and 5 (OSG3-OSG5). The free glucose is eliminated with baking yeast from the oligosaccharide mixture according to patent application FI 904124 or from the glucoamylase-treated mixture, whereby an oligosaccharide mixture lower in calories and less cariogenic than the untreated oligosaccharide mixture is obtained.

 The significance of after-treating the oligosaccharide mixture is based on the fact that it is pos-

sible by glucoamylase treatment to increase the yield of oligosaccharides with DP 3 to 5 (OSG3-OSG5). Such oligosaccharides can be used e.g. in the foodstuff industry as new raw materials because oligosaccharides with DP 3 to 5 are broken up very slowly, or not at all, by α amylase (e.g. in: Starch, Chemistry and Technology (1984), Eds. R.L. Whistler, J.N. BeMiller and E.F. Paschall, Academic Press Inc., London, England). Furthermore, removal of free glucose from the oligosaccharide mixture reduces the cariogenicity and calorie content of the mixture (e.g. in: Alternative sweeteners (1986), Eds. L.O. Nabors and R.C. Gelardi, Marcel Dekker Inc., New York, U.S.A., p. 185-216).

In the after-treatment procedure of the invention, the after-treatment may also be carried out with immobilized glucoamylase and/or yeast. Using the immobilizing technique it is possible to achieve a continuous after-treatment procedure, by which 30 to 60% of the cost can be saved, compared with conventional technique. Furthermore, the product is obtained in rather pure condition, which even further lowers the after-treatment cost and, on the other hand, makes possible new applications of the product.

The fundamental idea of the invention consists of the fact that glucoamylase has been found to break up α -(1-4) and α -(1-6)-glycosidic bonds in starch, producing glucose from starch. The oligosaccharide mixture of the patent application FI 904124 is starch-based, and the long glucose chains containing acceptor sugar (trehalose or cellobiose) can be broken up with glucoamylase to shorter units, the broken-up glucose chains decomposing further to glucose. By modifying the reaction conditions in controlled manner the yield of oligosaccharides with DP 3 to 5 can be increased. With prolonged reaction time and high glucoamylase concentration, the oligosaccharide mixture of patent application FI 904124 will decompose to glucose and acceptor

sugar. The glucose that has been set free in the oligosaccharide mixture can be removed with baking yeast, which is known to use glucose in its metabolism. The product thus obtained consists of acceptor sugar and an
5 oligosaccharide mixture containing, among others, oligosaccharides with DP 3-5.

The composition of the product mixtures was determined by liquid chromatography. The method enabled the concentrations of oligosaccharides under DP 8 to be
10 determined in that the concentration of any given oligosaccharide was calculated in accordance with the concentration of the reference standard (with DP 1 to 7) having the closest retention time. Confirmation of the results was made qualitatively with TLC.

15 Glucoamylase preparations of various origins can be used in the invention, such as commercial preparations, for instance. For yeast, e.g. *Saccharomyces cerevisiae* baking yeast may be used.

The concentration of the oligosaccharide mixture reacting with glucoamylase may be in the range
20 from 5 to 60%. The concentration may be high when soluble glucoamylase is used, while in the case of immobilized glucoamylase the favourable concentration range is 5 to 30%. For glucose elimination either free or
25 immobilized yeast cells may be used, and the concentration of the oligosaccharide mixture may then be in the range of 5 to 60%. The advantageous concentration range is 5 to 30%. The favourable pH range of the oligosaccharide mixture to be after-treated is 5.5 to 7.5. The
30 reactions may be effected with glucoamylase at 10 to 70°C, advantageous temperature is 30 to 50°C. For yeast, the temperature range may be 10 to 60°C, advantageous temperature is 30 to 45°C. In either case, the reaction time is 24 hrs at the most, advantageous reac-
35 tion time is 10 min. to 8 hrs, depending on the concentration of the glucoamylase or yeast used. The longer the reaction time with glucoamylase the more of the

OSG3-OSG5 oligosaccharides will the solution contain, compared with the untreated mixture. The solution contains the greatest amount of OSG3 oligosaccharide, next of OSG4 oligosaccharide, and so on, until at the turning point of the reaction the contents, first of OSG5 saccharide, then of OSG4 decrease as the chains are broken up. Ultimately the concentration of OSG3 oligosaccharide also begins to decline. The greater the quantity of oligosaccharides that are broken up the more glucose and acceptor sugar will the solution contain.

After-treatment with immobilized glucoamylase and/or yeast can be carried out either in batches or as a continuous column reaction. It is advisable, prior to continuous reaction, to filter the oligosaccharide mixture in order to remove any insoluble and unhydrolyzable starch. Baking yeast immobilized in alginate binds to itself some of the oligosaccharide contents in the mixture being after-treated, for which reason the continuous system should be allowed to stabilize until the yeast beads are saturated with the oligosaccharide mixture. Using a long retention time in the column and low immobilized yeast concentration will result in favourable conditions for the after-treatment.

Following upon the after-treatment of the invention, the oligosaccharide syrup may further be after-treated according to procedures known in the art, such as decolouring with active carbon and desalting with ion exchangers, whereafter the oligosaccharide syrup may be either cold-dried, powdered or concentrated in vacuum. Ethanol (e.g. 70%) may be used to precipitate the long-chain oligosaccharides and polysaccharides out of the mixture.

It is thus understood that in the after-treatment procedure of the invention oligosaccharide mixtures according to patent application FI 904124 can be modified in controlled manner, whereby mixtures are

obtained in which the proportion of oligosaccharides with DP 3 to 5 has been advantageously altered. In addition, it is possible with this after-treatment to achieve oligosaccharide mixtures lower in calories and less cariogenic than the untreated mixtures.

EXAMPLE 1

Yeast immobilization

The yeast cells were bonded to sodium alginate in the form of beads (Linko, Y-Y., Weckström, L. and Linko, P. (1980), Food Process Engineering, Vol. 2, p. 81-91). Baking yeast, 16.0 g wet weight, (Oy Alko Ab, Rajamäki) was suspended in 10 ml distilled water and added to 50 g 8% sodium alginate (BDH, Poole, England). The mixture was extruded with the aid of nitrogen gas to beads of 0.6 mm diameter through needles into 0.5 M calcium chloride solution, in which the beads were left to mix for 60 min. The beads were then washed with distilled water, and the excess of water was removed.

The activity of the immobilized yeast was determined using for substrate, 2% glucose solution (in 50 mM imidazole buffer, pH 6.8). 1.0 g of the immobilized yeast were added to 5 g substrate and the reaction was allowed to proceed at 40°C for 30 min. The concentration of residual glucose was determined at room temperature by liquid chromatography (Zsádon, B., Otta, K.H., Tudos, F. and Szejtli, J. (1979), J. Chromatogr., 172. 490-492). The elution rate in carbohydrate column was 0.9 ml/min, and the concentrations of the standards (with DP 1-7, including glucose, maltose, ..., maltoheptaose, cellobiose, trehalose) were 1-5 mg/l.

One unit of activity of the immobilized yeast beads is equivalent to 1 μ mol glucose per min. used up under the above reaction conditions. The activity of the immobilized yeast was found to be 7 U/g and the activity of free yeast cells under equivalent condi-

tions of reaction, 33.3 U/g. The activity yield of the immobilized yeast was found to be 100%.

EXAMPLE 2

5 Glucoamylase immobilization

The glucoamylase (Spezyme, Suomen Sokeri Oy, activity 340 GU/ml) was immobilized on Duolite ES 762 resin (Duolite International, Vitry, France). For im-
mobilization, to 50 mM imidazole buffer (pH 6.8) was
10 added suitably diluted glucoamylase 2 ml per g resin, and the enzyme was allowed to become linked to the resin under 16-hr shaking at room temperature. The en-
zyme solution was filtered off and 2.5% glutaric alde-
hyde was added 2 ml per g resin for cross-linking.
15 Shaking was continued for another 3 hrs at room temper-
ature, whereafter the resin was washed with distilled water and filtered to moist constitution.

The activity of the immobilized glucoamylase was determined using for substrate, 2% starch solution
20 (soluble starch in 50 mM imidazole buffer, pH 6.8). Im-
mobilized glucoamylase was added 0.5 g to a solution containing 4.5 g substrate and 0.5 ml distilled water. The reaction was allowed to proceed at 40°C for 30 min. The concentration of the glucose thus formed was deter-
25 mined by liquid chromatography (see Example 1). The activity of the soluble glucoamylase was determined by adding suitably diluted enzyme 0.5 ml to 4.5 g sub-
strate under equivalent conditions of reaction as above.

30 The unit of activity (U) of immobilized gluco-
amylase corresponds to 1 μ mol glucose produced in one minute under the above reaction conditions. The activ-
ity of the immobilized glucoamylase was found to be 3.6 U/g and that of the soluble enzyme, 378 U/ml. The
35 grade of activity of the immobilized glucoamylase was found to be 4.3%.

EXAMPLE 3After-treatment of oligosaccharide mixture with glucoamylase

Oligosaccharide mixtures according to patent application FI 904124 were after-treated with either soluble or immobilized glucoamylase preparation according to Example 2. To the oligosaccharide mixture, of which the composition is presented in Table 1 (trehalose oligosaccharides, dry matter content of the mixture 50%), was added soluble glucoamylase, either 1.1 U per g of mixture (Test 1) or 3.3 U per g of mixture (Test 2). Immobilized glucoamylase was added 0.11 U per g of mixture (Test 3). The batch reactions were allowed to proceed at 40°C under shaking for 3 hrs (Test 1), 5 hrs (Test 2) and 1.75 hrs (Test 3). The compositions of the products were determined by liquid chromatography (see Example 1), and they are given in Table 1.

A cellobiose oligosaccharide mixture was similarly after-treated, its composition being presented in Table 2 (dry matter content 30%). Soluble glucoamylase was added 0.67 U per g of mixture (Test 4) and immobilized glucoamylase, 0.22 U per g of mixture (Test 5). The batch reactions were allowed to proceed at 40°C under shaking for 3 hrs (Test 4) and 5 hrs (Test 5). The compositions of the products were determined by liquid chromatography (see Example 1), and they are given in Table 2.

The concentration of OSG3-OSG5 oligosaccharides could be increased by the glucoamylase treatment in all tests. The untreated oligosaccharide mixture presented in Table 1 had OSG3-OSG5 concentration 21.5 g/100 g, while the OSG3-OSG5 concentrations after glucoamylase treatment were 22.0-26.3 g/100 g. Especially in Test 2 the concentration of OSG3 oligosaccharide increased 85%, compared with the untreated mixture. Similarly, the untreated oligosaccharide mixture presented in Table 2 had OSG3-OSG5 concentration

11.2 g/100 g, while the OSG3-OSG5 concentrations after glucoamylase treatment were 12.3-14.3 g/100 g.

5 TABLE I. After-treatment of oligosaccharide mixture
with soluble and immobilized glucoamylase (Example 3).
The mixture was reacted with soluble (Test 1: 1.1 U
per g of mixture, and Test 2: 3.3 U per g of mixture)
or immobilized (Test 3: 0.11 U per g of mixture) gluco-
amylase at 40°C for 3, 5 and 1.75 hrs, respectively.
10 The concentrations of oligosaccharides (with DP less
than 8) were determined by liquid chromatography (see
Example 1).

15	Product	Native mixture	Test 1	Test 2	Test 3
		Concentration (g/100 g)			
20	Glucose	0.2	3.4	9.6	2.5
	Trehalose	10.5	12.2	14.5	13.5
	OSG3	9.1	13.0	16.8	13.6
	OSG4	7.1	8.4	4.5	8.3
	OSG5	5.3	3.9	0.7	4.4
	OSG6	3.5	1.8	0.6	2.4
25	OSG7	2.3	1.3	0.8	+
	OSG3-OSG5	21.5	25.3	22.0	26.3

TABLE II. After-treatment of oligosaccharide mixture with soluble and immobilized glucoamylase (Example 3). The mixture was reacted with soluble (Test 4: 0.67 U per g of mixture) or immobilized (Test 5: 0.22 U per g of mixture) glucoamylase at 40°C for 3 and 5 hrs, respectively. The concentrations of oligosaccharides (with DP less than 8) were determined by liquid chromatography (see Example 1).

	Native mixture	Test 1	Test 2
Product	Concentration (g/100 g)		
Glucose	-	2.9	5.6
Trehalose	5.9	7.5	8.8
OSG3	5.2	7.8	8.1
OSG4	3.3	4.5	2.3
OSG5	2.7	2.0	0.9
OSG6	1.9	0.6	-
OSG7	1.3	-	-
OSG3-OSG5	11.2	14.3	12.3

EXAMPLE 4

After-treatment of oligosaccharide mixture with yeast

An oligosaccharide mixture as in Test 1 presented in Example 3 was after-treated with free yeast cells as in Example 1, in a batch reaction, and an oligosaccharide mixture as in Example 3, with immobilized yeast cells as in Example 1, in a continuous reaction. The aim was to reduce the glucose concentration in the mixture.

To the oligosaccharide mixture of Test 1 free yeast cells were added 3.2 U per g of mixture and the reaction was allowed to proceed under shaking at 40°C for 1 hr, whereafter the yeast cells were filtered off. The composition of the product was determined by liquid chromatography (see Example 1) and is presented in

Table 3.

The oligosaccharide mixture of Test 3 was after-treated with immobilized yeast in a continuous column reaction at 40°C. Prior to glucose removal, the oligosaccharide mixture of Test 3 was diluted 1:10 with water, and filtered, whereafter the dry matter content of the solution was 5%. 20 g immobilized yeast (activity 7 U/g) were added into the column (1.8 cm diameter and 14 cm height), and the flow rate in the column was 0.8 ml/min. Collection of eluate was commenced upon stabilization of the column reaction. The composition of the eluate was determined by liquid chromatography (see Example 1) and is presented in Table 3.

It proved possible, with free yeast cells, to reduce the glucose concentration in the mixture 82%, and with immobilized yeast the glucose could be totally eliminated from the mixture. Fig. 1 displays the liquid chromatographic elution chromatogram of the oligosaccharide mixture of Test 3, after immobilized yeast treatment. The peaks numbered 1 to 6 in the chromatogram are: 1= trehalose, 2= OSG3, 3= OSG4, 4= OSG5, 5= OSG6, and 6= OSG7.

TABLE III. After-treatment with free yeast cells (3.2 U per g of mixture, reaction time 1 hr) of the oligosaccharide mixture of Test 1, presented in Example 3, and after-treatment with immobilized yeast in a column (column activity 140 U, flow rate 0.8 ml/min) of the oligosaccharide mixture of Test 3, diluted to 5% dry matter content, at 40°C (Example 4). The oligosaccharide (DP less than 8) concentrations were determined by liquid chromatography (see Example 1).

	Concentration (g/100 g)	
	Free yeast cells	Immobilized yeast cells
Product		
Glucose	0.6	-
Trehalose	11.9	1.07
OSG3	12.8	1.26
OSG4	8.6	0.77
OSG5	4.1	0.38
OSG6	1.9	0.19
OSG7	1.3	+
OSG3-OSG5	25.5	0.241

CLAIMS

1. A procedure for modifying an oligosaccharide mixture, characterized in that free
5 or immobilized glucoamylase and/or yeast is made to act on an oligosaccharide mixture which has been obtained by making cyclomaltodextrin-glucoamyltransferase (CGTase, E.C. 2.4.1.19) act on starch in the presence of acceptor sugar.
- 10 2. Procedure according to claim 1, characterized in that glucoamylase is made to act on the oligosaccharide mixture, whereby the glucoamylase breaks up the long-chain oligosaccharides to shorter units, at the same time setting free glucose, whereby
15 the proportion in the mixture of oligosaccharides with DP from 3 to 5 can be modified.
3. Procedure according to claim 1, characterized in that yeast is made to act on the oligosaccharide mixture, whereby the yeast will use the
20 free glucose in the mixture towards its metabolism, whereby an oligosaccharide mixture lower in calories and less cariogenic is obtained.
4. Procedure according to claim 1, characterized in that glucoamylase and yeast are
25 made to act on the oligosaccharide mixture, either in steps or concurrently, whereby the glucoamylase breaks up the long-chain oligosaccharides to shorter units and the yeast uses towards its metabolism the glucose that
30 has been set free in the solution, whereby an oligosaccharide mixture lower in calories and less cariogenic is obtained in which the proportion of oligosaccharides with DP from 3 to 5 has been modified.
5. A starch-based oligosaccharide mixture manufactured by a procedure according to any one of
35 claims 1-4, characterized in that it contains 0-100%, advantageously 85-100%, less OSG7 and OSG6, and 0-100%, advantageously 97-100%, less glucose

than the untreated oligosaccharide mixture and 0-55% more OSG5, 0-75% more OSG4, 0-90% more OSG3 and 0-15% more acceptor sugar than the untreated oligosaccharide mixture.

5 6. Starch-based oligosaccharide mixture manufactured by a procedure according to claim 2, characterized in that it contains glucose 0-35%, acceptor sugar 3-85%, OSG3 1-60%, OSG4 1-35%, OSG5 0.5-25%, OSG6 0-15% and OSG7 0-10%, calculated on the dry
10 matter of the solution.

 7. Starch-based oligosaccharide mixture manufactured by a procedure according to claim 3, characterized in that it contains glucose 0-3%, acceptor sugar 7-75%, OSG3 1.8-32%, OSG4 3-20%, OSG5
15 2.5-16%, OSG6 0.8-12% and OSG7 0.2-10%, calculated on the dry matter of the solution.

 8. Starch-based oligosaccharide mixture manufactured by a procedure according to claim 4, characterized in that it contains glucose 0-3%, acceptor sugar 3-85%, OSG3 1-60%, OSG4 1-35%, OSG5 0.5-25%, OSG6 0-15% and OSG7 0-10%, calculated on the dry
20 matter of the solution.

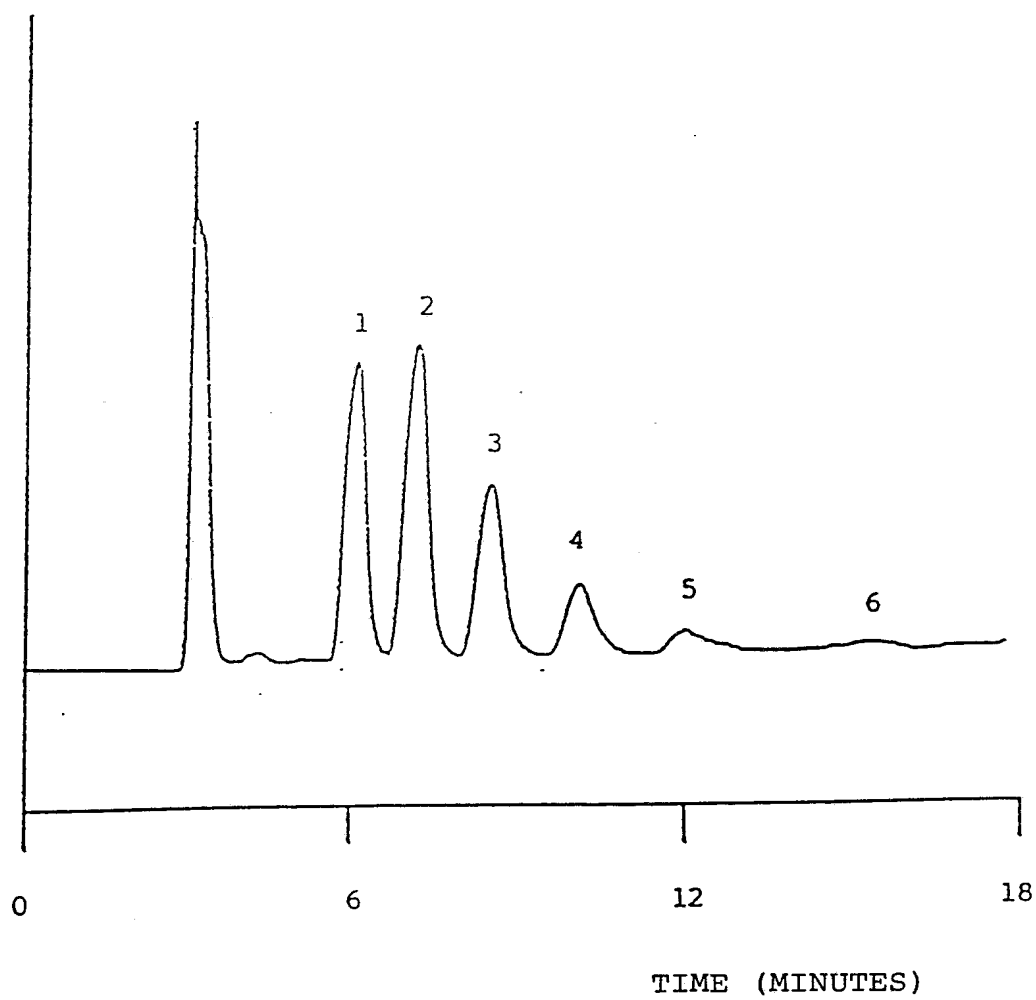


FIG. 1

INTERNATIONAL SEARCH REPORT

International Application No PCT/FI 91/00240

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶ According to International Patent Classification (IPC) or to both National Classification and IPC IPC5: C 12 P 19/20, 19/18														
II. FIELDS SEARCHED <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black;">Minimum Documentation Searched⁷</div> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 25%; border: 1px solid black;">Classification System</th> <th style="border: 1px solid black;">Classification Symbols</th> </tr> <tr> <td style="border: 1px solid black; height: 40px; vertical-align: bottom;">IPC5</td> <td style="border: 1px solid black; vertical-align: bottom;">C 12 P</td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched⁸</div> <p style="padding: 5px;">SE,DK,FI,NO classes as above</p>			Classification System	Classification Symbols	IPC5	C 12 P								
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IPC5	C 12 P													
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹ <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 10%; border: 1px solid black;">Category *</th> <th style="width: 70%; border: 1px solid black;">Citation of Document,¹¹ with indication, where appropriate, of the relevant passages¹²</th> <th style="width: 20%; border: 1px solid black;">Relevant to Claim No.¹³</th> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top;">X</td> <td style="border: 1px solid black; vertical-align: top;">EP, A2, 0252525 (WAKO PURE CHEMICAL INDUSTRIES, LTD.) 13 January 1988, see especially claim 4 --</td> <td style="border: 1px solid black; text-align: center; vertical-align: top;">1-8</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top;">Y</td> <td style="border: 1px solid black; vertical-align: top;">Chemical Abstracts, volume 109, no. 3, 18 July 1988, (Columbus, Ohio, US), see page 655, abstract 23312m, & JP, A, 6317895 (Preparation of new gluco-oligosaccharide derivatives and their use for determining alfa-amylase activity) 25 January 1988 --</td> <td style="border: 1px solid black; text-align: center; vertical-align: top;">1-8</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top;">A</td> <td style="border: 1px solid black; vertical-align: top;">EP, A2, 0164933 (CPC INTERNATIONAL INC.) 18 December 1985, see especially claims 1 and 4 --</td> <td style="border: 1px solid black; text-align: center; vertical-align: top;">1-8</td> </tr> </table>			Category *	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³	X	EP, A2, 0252525 (WAKO PURE CHEMICAL INDUSTRIES, LTD.) 13 January 1988, see especially claim 4 --	1-8	Y	Chemical Abstracts, volume 109, no. 3, 18 July 1988, (Columbus, Ohio, US), see page 655, abstract 23312m, & JP, A, 6317895 (Preparation of new gluco-oligosaccharide derivatives and their use for determining alfa-amylase activity) 25 January 1988 --	1-8	A	EP, A2, 0164933 (CPC INTERNATIONAL INC.) 18 December 1985, see especially claims 1 and 4 --	1-8
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Y	Chemical Abstracts, volume 109, no. 3, 18 July 1988, (Columbus, Ohio, US), see page 655, abstract 23312m, & JP, A, 6317895 (Preparation of new gluco-oligosaccharide derivatives and their use for determining alfa-amylase activity) 25 January 1988 --	1-8												
A	EP, A2, 0164933 (CPC INTERNATIONAL INC.) 18 December 1985, see especially claims 1 and 4 --	1-8												
<div style="display: flex; justify-content: space-between;"> <div style="width: 48%;"> <p>* Special categories of cited documents:¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 48%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>														
IV. CERTIFICATION <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; border: 1px solid black; padding: 5px;"> Date of the Actual Completion of the International Search <div style="text-align: center; font-weight: bold;">4th February 1992</div> </td> <td style="width: 50%; border: 1px solid black; padding: 5px;"> Date of Mailing of this International Search Report <div style="text-align: center; font-weight: bold;">1992 -02- 0 6</div> </td> </tr> <tr> <td style="border: 1px solid black; padding: 5px;"> International Searching Authority <div style="text-align: center; font-weight: bold;">SWEDISH PATENT OFFICE</div> </td> <td style="border: 1px solid black; padding: 5px;"> Signature of Authorized Officer <div style="text-align: center;"> <i>Carolina Palmcrantz</i> Carolina Palmcrantz </div> </td> </tr> </table>			Date of the Actual Completion of the International Search <div style="text-align: center; font-weight: bold;">4th February 1992</div>	Date of Mailing of this International Search Report <div style="text-align: center; font-weight: bold;">1992 -02- 0 6</div>	International Searching Authority <div style="text-align: center; font-weight: bold;">SWEDISH PATENT OFFICE</div>	Signature of Authorized Officer <div style="text-align: center;"> <i>Carolina Palmcrantz</i> Carolina Palmcrantz </div>								
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International Searching Authority <div style="text-align: center; font-weight: bold;">SWEDISH PATENT OFFICE</div>	Signature of Authorized Officer <div style="text-align: center;"> <i>Carolina Palmcrantz</i> Carolina Palmcrantz </div>													

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	GB, A, 2165549 (DIRECTOR OF NATIONAL FOOD RESEARCH INSTITUTE MINISTRY OF AGRICULTURE FORESTRY AND FISHERS) 16 April 1986, see especially claims 7-8 and page 2 lines 1-6 --	1-8
Y	WO, A1, 8907148 (BIOEUROPE) 10 August 1989, see especially claims 8 and 9 --	1-8
Y	Dialog Information Services, file 351, WPI, 81-91, Dialog Acc. No. 007662427, ((NORQ) NORINSHO), "Neotrehalose and centose prepn. - by treating starch substrate with cyclodextrin - synthetase; GLUCOSYL MALTOSE; JP 63216492, A, 880908, 8842 --	1-8
Y	US, A, 4477568 (HENDRIK HOKSE ET AL) 16 October 1984, see especially claim 1 --	1-8
Y	Chemical Abstracts, volume 114, no. 15, 15 April 1991, (Columbus, Ohio, US), see, abstract 141647a, & JP, A, 2255095 (Effective production of γ -cyclodextrin and/or alfa-glucosylglycyrrhizin with cyclodextrin glucanotransferase) 15 October 1990 -- -----	1-8

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. PCT/FI 91/00240**

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the Swedish Patent Office EDP file on 30/11/91. The Swedish Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A2- 0252525	88-01-13	JP-A- 63301892	88-12-08
		JP-A- 63170393	88-07-14
EP-A2- 0164933	85-12-18	AU-B- 585860	89-06-29
		AU-D- 4313385	85-12-12
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		JP-A- 61019498	86-01-28
		US-A- 4650757	87-03-17
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		JP-A- 61092592	86-05-10
		US-A- 4668626	87-05-26
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		FR-A-B- 2626583	89-08-04
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		NL-A- 8104410	83-04-18